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The Urinary Nitrogen Distribution of Representative Members of the Carnivora.

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(Text-figures 1-5).

One of the many interesting chapters in our knowledge of comparative biochemistry is that dealing with the form in which nitrogen is excreted from the animal body. An excellent perspective of a considerable number of the quantitative investigations in this field is provided in a table compiled by Needham (1931, pp. 1139-41). With few exceptions among both the invertebrates and vertebrates, ammonia, urea and uric acid have been shown to account for the bulk of the nitrogen eliminated, and according to Needham (1931, p. 1132), these three compounds appear to be the only substances "which are available in the animal kingdom for carrying away the nitrogenous waste resulting from protein breakdown." There are to be found in the urine, of course, quite a number of other nitrogenous constituents, but their combined nitrogen content is usually only a small fraction of the total nitrogen. Baldwin has summarized the salient facts concerning the excretory products of protein and purine metabolism in vertebrates as shown in Table I.

The fascinating evolutionary aspects of the biochemical differences briefly surveyed above, though outside the province of this paper, have been dealt with in several contributions (cf. Needham (1929; 1931, p. 1132), Smith (1932; 1935), Baldwin (1937) and Florkin (1935)).

The mammalian class exhibits a monotonous regularity in the employment of urea as the chief end-product of nitrogen metabolism. This holds even for the egg-laying mammal, *Echidna aculeata* (Neumeister (1898); Robertson (1923); Mitchell (1931)). One variation of note in the urinary nitrogen partition pertains to the excretion of hippuric acid. Though the synthesis of this substance is by no means limited to the herbivorous animals, the latter, after ingesting large amounts of hay and other benzoic acid-yielding feed, may excrete a considerable fraction of the total nitrogen as hippuric acid. Again, the diet of the herbivora is often predominantly base-forming and this leads apparently to a diminished production and elimination of nitrogen in the form of ammonium salts. A variation of more fundamental significance, inasmuch as it is related to specific metabolic processes rather than to dietary habits, is that evinced by various mammalian species in the extent of oxidation of the purine bodies before elimina-

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TABLE I.
After Baldwin (1937, p. 61).†

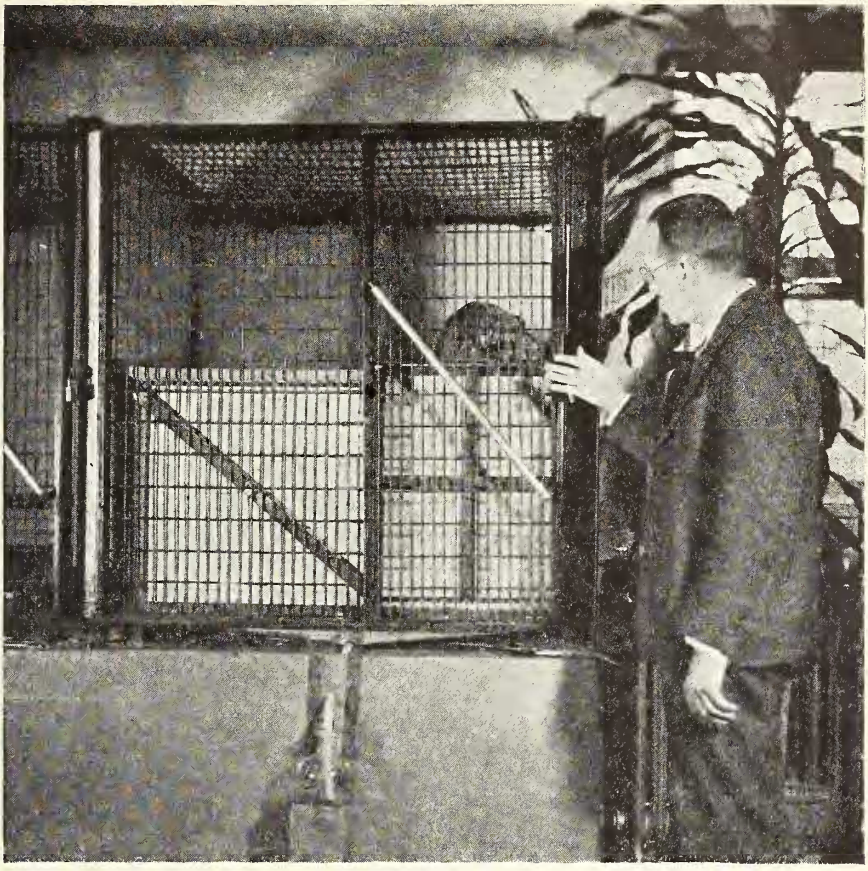
	End-product of	
	Protein metabolism	*Purine metabolism*
Mammalia	Urea	Allantoin‡
Aves	Uric acid	Uric acid
Reptilia:		
Snakes, lizards	Uric acid	Uric acid
Turtles	Urea	Allantoin ?
Amphibia	Urea	Urea
Pisces		
Elasmobranchii	Urea	Urea
Teleostei	Ammonia	Urea

† From "An Introduction to Comparative Biochemistry," by Ernest Baldwin, by permission of Cambridge University Press, London.
‡ Uric acid in man, higher apes and Dalmatian dog.

tion. Man, the anthropoid apes, and to a degree the Dalmatian dog, excrete uric acid as an end-product of purine metabolism whereas the remaining mammalian forms, as far as data are available to show, carry the oxidation a stage further to allantoin. With regard to the excretion of creatinine and creatine, Hunter (1928, pp. 104-5) states that creatinine is never absent from the urine of mammals and further that "it is probable . . . that every adult mammal, when placed under standard conditions upon a creatine-free diet, excretes only creatinine."

The nitrogen distribution in the urine of the order Carnivora, as represented by the domestic cat and dog, conforms to what has been said above concerning the general character of mammalian urine. Urea is the dominant nitrogen-containing component, and the main product of purine metabolism is allantoin. Studies of the nitrogenous substances in the urine of some of the wild members of the Carnivora date back to the early part of the nineteenth century (see Milne Edwards (1862)) but these studies naturally were limited in scope and accuracy. Investigations dealing with non-domesticated Carnivora and involving the use of modern methods of urine analysis, insofar as revealed by our search of the literature, are as follows: of the coyote by Swain (1905) and by Hunter & Givens (1910-11); of the fox and coyote by Hawk (1910-11); of the weasel, raccoon dog (*Nyctereutes viverrinus*), tiger, leopard and hyena by Fuse (1925); and of the seal by Smith (1936)[1]. Also, Hunter and associates (1914 and 1920) showed that the uricolytic index[2] of the raccoon, black bear, badger, coyote and dingo, as of the domestic cat and dog, is high, the excretion of allantoin being in large excess over that of uric acid. Without going further into a detailed analysis of these contributions, it may be stated that they all point to a similar pattern in the urinary nitrogen distribution of the Carnivora. In contrast to this picture of uniformity was the finding of S. R. Benedict (1916) that the pure strain Dalmatian coach dog excretes an unusual amount of its purine end-product as uric acid. The uricolytic index is in

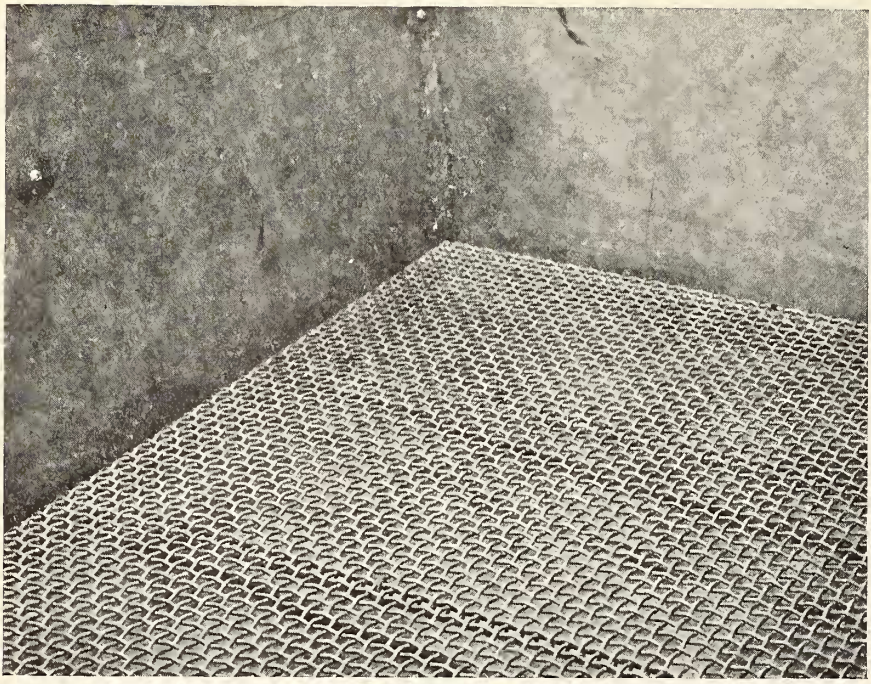
[1] Swain & Rakestraw (1923) reported the presence of uric acid in the urine of the sea lion.
[2] This term is defined on a subsequent page.



Text-figure 1.
Exterior view of metabolism cage.

the neighborhood of 30-40, intermediate between the much higher values found for other Carnivora and the very low figures in the case of man and anthropoid apes. This rather strange aberration in an otherwise fairly uniform series indicates that it is at least possible that other similar instances of significant variation in urinary nitrogen partition may await detection—even among the different families and species of one order as, for example, the Carnivora.

We were therefore interested, when the opportunity arose in connection with experiments initiated for the purpose of studying the kynurenic acid excretion by representative species of the Carnivora, in determining as well the nitrogen distribution of the urines of these animals. In our work, analyses were made for total, urea, ammonia, creatinine and creatine nitrogen, and as far as facilities would permit, for allantoin and uric acid nitrogen. The volume of urine voided in a given period, generally 24 or 48 hours, and the specific gravity of each sample, have a'so been recorded. As will be seen, some of the species included in our study have been investigated before. However not all of the previous analyses were made on samples collected over a definite period, and in some cases the analyses were not sufficiently extensive to account for the most of the urinary nitrogen.

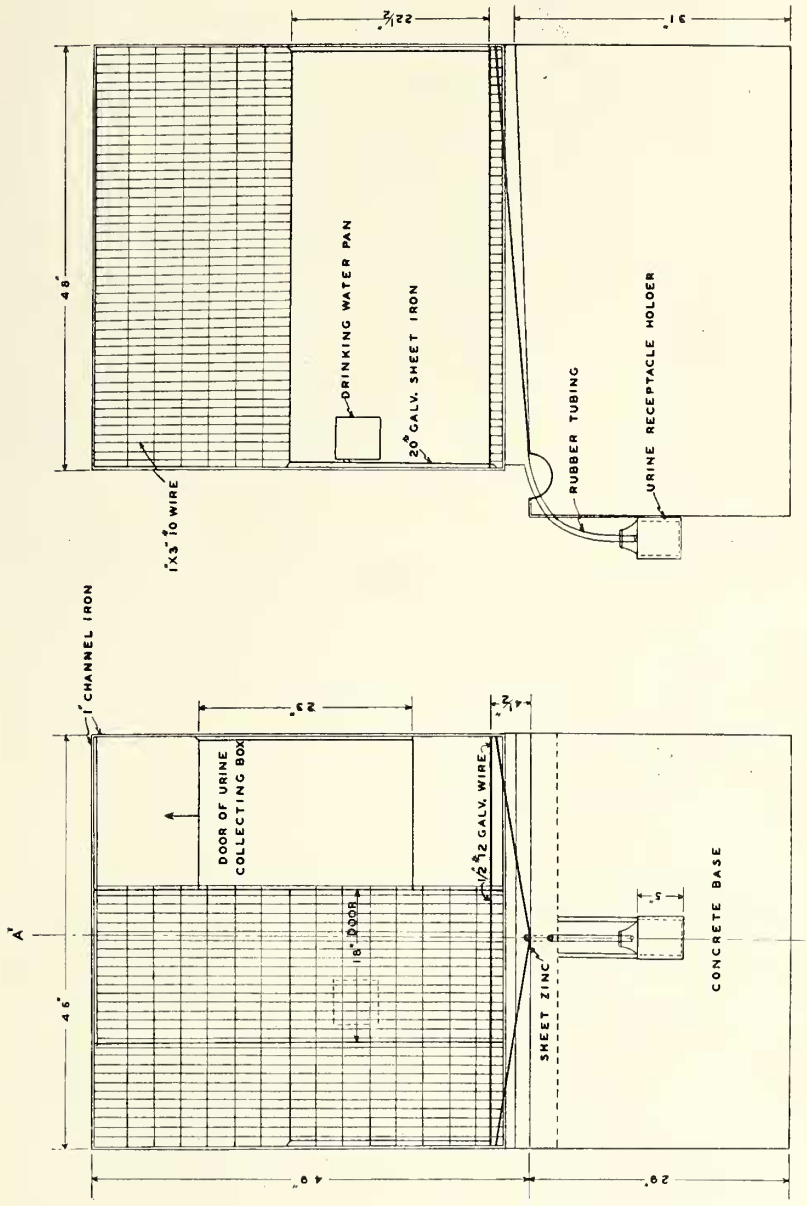


Text-figure 2.
Interior view of metabolism cage.

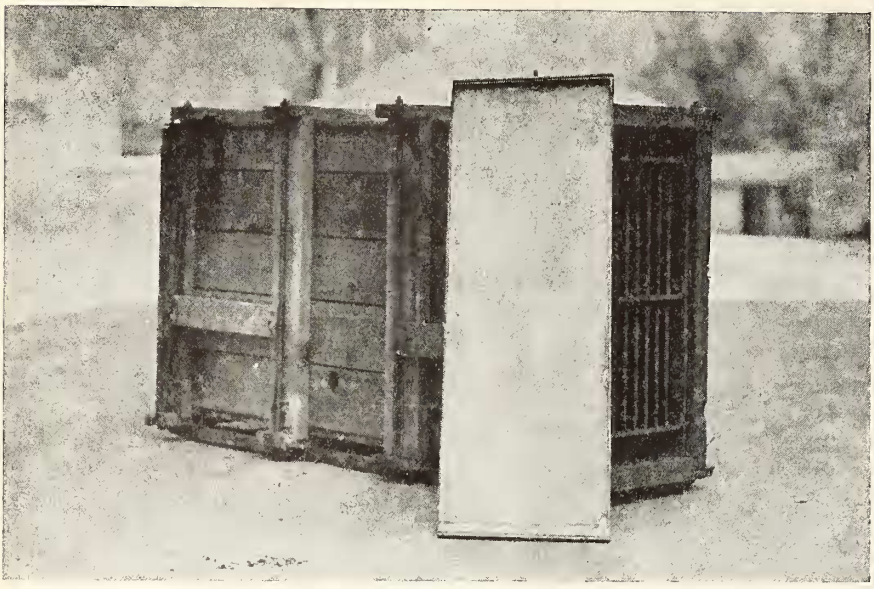
Experimental.

For the collection of urine, the genets and the skunk were confined in a small metabolism cage of the type ordinarily employed with rabbits. For the remainder of the animal subjects, excepting the bear, a special cage was constructed by modifying one of the cages available at the Zoological Park Hospital. The original cage is about 4 feet along each edge and is constructed of heavy wire mesh mounted on angle iron and is bolted to an elevated concrete floor. The alteration of this cage for our purpose was accomplished by building and inserting a snugly fitting unit consisting of a urine collecting box with deep side walls to insure against loss of urine, and with a heavy false bottom to protect the lighter solid zinc metal bottom below and to hold back fecal material. The false bottom in position rests on lugs but may be raised free of these and, if desired, removed from the cage, to facilitate cleaning. All seams of the urine collecting box are soldered so that except for the door it is water tight. The door, like the sidewalls, is constructed of galvanized iron. It is inserted vertically through channels in the adjacent side walls and extends to a point below the door sill and also below the false bottom. This door is contiguous with the door of the cage proper. The complete metabolism cage is illustrated in Text-figs. 1, 2 and 3. It has served excellently for animals ranging in size from the raccoon and fox to the cheetah and hyena.

In order to collect urine from the Tibetan bear, it was necessary to prepare still another cage (see Text-figs. 4 and 5). This was effected by placing a close-fitting, shallow, flat, galvanized iron pan on the bottom of a heavily constructed crate ordinarily used for shipping bears. This pan extends from the rear of the crate about three-quarters of the way forward



Text-figure 3.
Schematic drawing of metabolism cage.

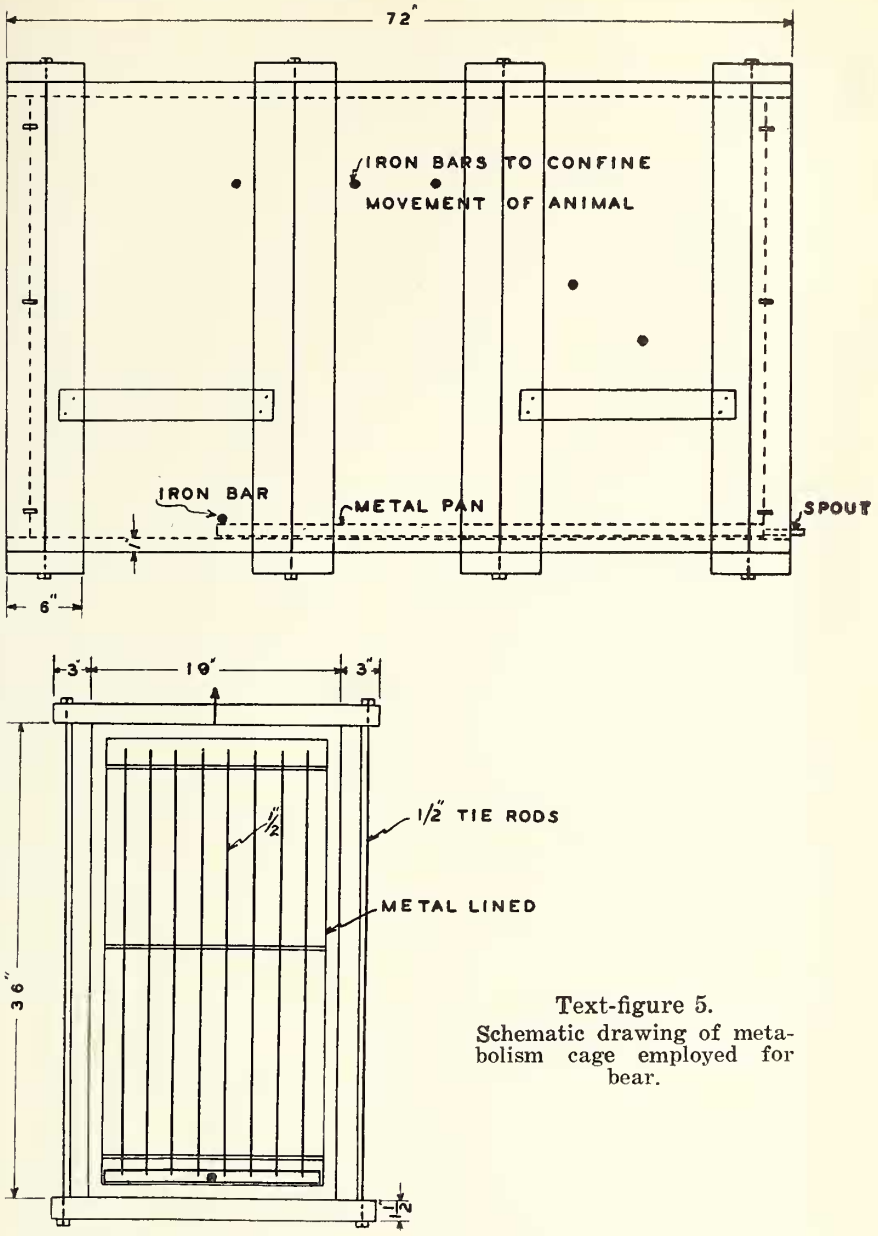


Text-figure 4.
Photograph of cage with pan, used in metabolism experiments on bear.

so that with a male animal no urine is lost. In use, the cage is placed on blocks with the front end slightly elevated thus to promote the flow of the urine to the rear of the pan through the tubulature to the collecting bottle. By having the urine collecting pan of such length as not to extend forward beneath the head of the bear, it is possible to supply water periodically without danger of its being spilled into the urine. The predilection of our bear subject for using his front paws to bend up the front end of the urine-collecting pan was thwarted by inserting a heavy iron bar laterally through the cage and just over the front end of the pan.

All of the animals studied by us were ingesting diets composed entirely or largely of meat or of fish. Water was supplied *ad libitum* unless the individual exhibited a persistent tendency to dislocate the water container with danger of diluting the urine. In this event, drinking water was given at intervals during the day. The sea lion was generally permitted to secure his supply of water from his generous daily quota of fresh (frozen) fish. However, in order to guard against physical discomfort of the sea lion while under experiment, the animal was occasionally moistened with a fine mist of water from an insect gun, or wet sheets were hung about the cage.

All specimens of urine, with the exception of one which was secured from the bladder at autopsy, were collected from the metabolism cages directly into bottles containing toluene. The samples were filtered at room temperature to remove any hair or occasional slight contamination of fecal material, and then immediately stored under toluene at a temperature of 5° C. preliminary to analysis. The analytical procedures employed were as follows: total nitrogen by the Kjeldahl method; urea plus ammonia nitrogen by the urease-aeration-titration procedure of Van Slyke & Cullen (1914 and 1916); ammonia by the method of Folin & Bell (1917); creatinine by Folin's colorimetric method (see Hawk & Bergeim (1937)); creatine according to Benedict (1914); allantoin by Larson's method (1932); and uric acid by the indirect precipitation method of Benedict & Hitchcock



Text-figure 5.
Schematic drawing of metabolism cage employed for bear.

(1915). With the advice of Dr. Benedict, we incorporated two modifications of the procedure for determination of uric acid. The arsenophosphotungstate reagent of Benedict (1922) was substituted for that of Folin & Denis, and 15% of urea was added to the sodium carbonate solution to prevent turbidity (cf. Folin (1930) and Christman & Ravwitch (1932)). Acidity of the urines was tested with litmus paper, and the specific gravity was determined by means of a urinometer.

Discussion.

The results of our experiments on fifteen different species representing eight different families of the Carnivora are presented in Tables II and III. It is to be emphasized that the information given relative to the diets is, as stated, a rough estimate. The food was ordinarily not weighed and the amount of food intake was therefore subject to fluctuation. The diet compositions show nevertheless that all the animals were on a comparable basis in the ingestion of a high-protein diet. In connection with a study of kynurenic acid excretion to be reported elsewhere, tryptophane was administered in some of the experiments. Inasmuch as this procedure did not appear to alter the distribution of nitrogen, these experiments have been included in the tables. Again, it should be pointed out that the figures for urine volume and total nitrogen are for the stated collection period. In the case of the genet, for example, the values (Table II) are for two animals over a period of 45 hours. Furthermore, the urine volumes are those collected from the cage and cannot be taken as necessarily representing the exact amounts of urine produced during the experimental periods. The urines with few exceptions were acid to litmus. The exceptions were doubtless the result of some conversion of urea to ammonia despite the precautions taken to prevent bacterial action.

The distribution of nitrogen is much the same for all species studied by us, and is generally similar to that reported in previous experiments on members of the Carnivora. The majority of the values for the different nitrogenous constituents, expressed as nitrogen in per cent. of total nitrogen, fall within the following ranges: urea, 80 to 86, ammonia, 2 to 5, urea plus ammonia, 83 to 89, creatinine, 1 to 2, creatine, 1.5 to 3, allantoin, 2 to 4, uric acid, 0.05 to 0.20. The extent of deviation from these ranges may be seen by inspection of the tables. The few values for ammonia nitrogen which are over 7% of the total nitrogen are very likely the result of some bacterial conversion of urea, inasmuch as either the urine was actually alkaline (Exp. 37, Table II) or there had been opportunity for soiling of the cage during the immediately preceding collection periods (Exps. 33a, 29a, 35c, and 32a, Table III). A part of our program was carried out during warm weather which, of course, would be especially conducive to ammonia production. However, in no instance was there evidence of any extensive decomposition of the urine specimen. The relatively low percentages for urea and the corresponding elevated values for some of the other constituents in the bear experiments (Table III) may owe their explanation to a relatively lower nitrogen intake or to the storage of nitrogen.

The uricolytic index, that is, the per cent. allantoin nitrogen of total allantoin and uric acid nitrogen excreted, was determined by Hunter and his co-workers on animals which were either fasting or ingesting a diet low in purines. The purpose, of course, was to confine the criterion to the endogenous purine metabolism and thereby to eliminate the variable and disturbing influence of the exogenous metabolism of purines in the diet. Nevertheless, Hunter & Givens (1910-11) in their early studies on the coyote found that when the animal was ingesting a meat diet supplying relatively considerable quantities of purine material, the allantoin nitrogen constituted more than 95% of the total allantoin and purine (including uric acid) nitrogen excreted. The authors state, "Whether, therefore, endogenous or exogenous purines be concerned, it is evident that among the end-products of their metabolism allantoin plays an enormously preponderating part." What Hunter & Givens report in regard to the allantoin-uric acid relationship for the coyote ingesting a meat diet, we have found essentially to be true also for the fox, dingo, cheetah, serval, civet, badger, Tibetan and grizzly bears, raccoon and sea lion, all likewise ingesting a diet wholly or mainly of flesh. In the case of the grizzly bear, the allantoin nitrogen constituted 84% of the total allantoin and uric acid nitrogen excreted, in the other cases, the values were 92% or higher. Fuse found a similar pre-

anidae).

Family, common name, species	*tion of N in per cent of total N*				
	inine	*Creatine N*	*Allantoin N*	*Uric acid N*	*Undetermined N*
Felidae					
Cheetah (*Acinonyx jubatus*)	7	1.3	2.3	0.06	9.7
Cheetah (same animal)	6	2.3	2.2	0.07	10.9
Serval (*Felis serval*)	7	1.4	2.0	0.08	5.0
Serval (*Felis serval*)	5	2.0		0.04	
Viverridae					
Civet (*Civettictis civetta*)	3	0.6	2.4	0.04	5.4
Civet (same animal)		1.7	2.8	trace	3.1
Civet (*Civettictis civetta*)	5	2.2			
Genet 2 animals (*Genetta ludia*)	total = 2.5				
Hyaenidae					
Hyena (*Hyaena hyaena*)		1.9		0.056	
Hyena (same animal)		1.8		0.054	
Canidae					
Coyote (*Canis latrans*)	0	1.0	1.4	0.13	7.2
Coyote (same animal)	3	1.4	1.6	0.12	7.1
Red fox (*Vulpes fulva*)		2.1	2.2	0.16	6.5
Red fox (same animal)		2.2	2.0	0.11	10.7
Dingo (*Canis dingo*)		1.4	3.2	0.12	4.5
Wolf (*Canis nubilus*)		2.5			
Wolf (same animal)					

† Specimens not fully

* The symbol T is for gm. given during the entire period whether 24 or 48 hours, etc.